

**Technical Documentation to Support Development of  
Minimum Flows and Levels for the Caloosahatchee  
River and Estuary**

**Appendix D**

**Salinity Tolerance of *Vallisneria* and Salinity Criteria**

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## Salinity Tolerance of *Vallisneria* and Salinity Criteria

### Introduction

The Caloosahatchee MFL rule includes two salinity criteria. An exceedance occurs (1) if the 30-day moving average salinity at Ft Myers exceeds 10 ppt and (2) if a single daily average salinity exceeds 20 ppt. The research program initiated in response to the scientific peer review has generated new laboratory and field data on the salinity tolerance of *V. americana*. In this report data are analyzed to determine if these threshold salinities are still supported. The technical criteria also state that a flow of 300 cfs is necessary to maintain *Vallisneria* in the upper estuary. Data from our field monitoring program are analyzed to determine if this flow is supported.

### Methods

#### Salinity Tolerance of *Vallisneria*

Salinity tolerance was determined both from an analysis of monitoring of field populations and from laboratory mesocosm experiments designed to measure the effects of salinity on growth and mortality. Detailed descriptions of laboratory methods may be found in Doering et al. 1999, Doering and Chamberlain 2000, and Doering et al. 2001. A brief description appears below.

*V. americana* collected from the Caloosahatchee, was planted in rectangular tubs (14 cm H x 24 cm L x 15 cm W) containing sediment from the site of collection. Initially, tubs contained 4 to 8 shoots. Tubs and plants were distributed among ten cylindrical mesocosm tanks (1.3 m in diameter x 1 m deep, n=4 to 6 tubs/tank depending on the experiment) filled with water to a depth of 60 cm (volume=800 l). The tanks were located indoors at an aquarium facility at the Gumbo Limbo Nature Center in Boca Raton, FL. A 1000 Watt metal halide lamp, kept on a L:D photoperiod of 12:12 h, supplied light to each tank.

A given salinity was maintained by mixing appropriate volumes of fresh and salt water (total volume=114 l) from each of two head tanks located above each mesocosm. Head tanks were alternately filled and emptied into the mesocosms using solenoid valves controlled by timers. Thus, water was delivered to the mesocosms in a series of 114-liter pulses. Water in the mesocosms was replaced 3 times daily. Salt water was pumped from the Atlantic Ocean. Tap water, passed through a series of activated charcoal towers and filters (20 micron pore size) to remove chlorine, was used as a source of fresh water.

The net growth data summarized in this report was collected during 4 experiments conducted between 1996 and 2001 (**Table D-1**). Plants were exposed to constant salinity treatments (n=two mesocosms per treatment) for periods of 3 to 10 weeks. The exact salinity treatments depended on whether the purpose of the experiment was to measure growth at low salinity or tolerance to high salinity. Numbers of blades and shoots were counted on a weekly basis. These weekly measurements were used to determine net (production – loss) rates of blade and shoot growth. Data taken on a given day in each mesocosm were averaged across tubs, yielding two data points per treatment per week of the experiment. Net growth was modeled using the exponential growth equation ( $N_t = N_0 e^{rt}$ ) where  $N_0$  is the number of blades or shoots at the beginning of exposure and  $N_t$  is the number after  $t$  days.

A program to monitor *V. americana* on a monthly basis at Stations 1 - 4 (**Figure D-1**) was instituted in 1998. At each station, a pair of 100-m transects (one parallel to shore, one perpendicular to shore) was established at two sites. On each sampling date the number of blades and shoots was counted in 5 0.1 m<sup>2</sup> quadrats placed randomly along each transect (n=5 quadrats per transect, n=10 quadrats per site, n=20 quadrats per station). A detailed analysis of the first year of data may be found in Bortone and Turpin (2000). Data reported here encompass the time period January 1998 – April 2001.

Both field and laboratory data were used to select tolerable salinity thresholds that could be used to calculate minimum and maximum flows (see above). Field salinity tolerances were identified from plots of plant density as a function of salinity on the day of sampling. Tolerable, threshold salinities were those associated with marked or rapid

changes in plant density. Laboratory data were examined to identify a range of salinity where growth was low, and close to zero. Using non-linearities to identify thresholds is common in methods used to determine flow requirements for streams and rivers (Estevez 2000b).

## **Results**

### **Salinity Tolerance of *Vallisneria***

For *V. americana*, the net growth rates of shoots and blades in the laboratory decreased as salinity increased, with mortality occurring at salinities greater than 15 o/oo (**Figure D-2**). At 18 o/oo a 50% loss of shoots would occur in 38 days. At 20 o/oo a 50% loss would occur in 16 days. In the region between 10 o/oo and 15 o/oo, the change in growth in response to a change in salinity was very small. This lack of response was especially evident for the number of blades: growth rates at 10 o/oo and 15 o/oo were virtually identical. In this zone, plants survived but net growth rates of shoots and blades were low and relatively unchanging.

Data from field monitoring agreed well with results from the laboratory (**Figure D-3**, upper panel). The distribution of points in the four quadrants defined by the reference lines on the graph of shoot density and salinity may be treated as cells in a 2 x 2 contingency table. Higher densities in the field ( $> 400$  shoots  $m^{-2}$ ) occurred at salinities less than about 10 o/oo. Lower densities ( $< 400$  shoots  $m^{-2}$ ) were more frequent at salinities above 10 o/oo (**Figure D-3**, upper panel,  $X^2 = 4.53$ ,  $p < 0.05$ ).

### **Effect of Discharge on Density of Plants in the Field**

To investigate the potential effects of discharge on *Vallisneria* in the upper estuary, plant density data collected during the monitoring program was plotted as a function of the average discharge at S-79 for the 30 days prior to sampling. Field monitoring data indicated that higher shoot densities ( $> 400$   $m^{-2}$ ) occur at 30-day average discharges greater than  $8.5$   $m^3$   $sec^{-1}$  (300 cfs) (**Figure D-5**,  $X^2 = 7.98$ ,  $p < 0.01$ ).

## Discussion

The use of marine and estuarine SAV for management purposes appears to be a well-accepted practice (Batiuk et al. 1992; Dennison et al. 1993; Stevenson et al. 1993; Tomasko et al. 1996; Johansson and Greening 2000; Virnstein and Morris 2000). This stems in part from their sensitivity to pollutants of interest (e.g. nutrients, Tomasko et al. 1996) and in part from their ecological significance.

Although *V. americana* is considered a freshwater species, it is salt tolerant. Salinity tolerances of *V. americana* reported in the literature vary (Doering et al. 1999). Bourn (1932; 1934) found that growth declined with increasing salinity until it ceased at 8.4 ‰. Haller et al. (1974) reported growth at 3.33 ‰, no growth at 6.66 ‰ or 10 ‰ and death at 13.3 ‰. After 5 weeks, Twilley and Barko (1990) found no effect of salinity on growth over the range 0 ‰ to 12 ‰. Our laboratory results suggest that for V. americana from the Caloosahatchee, growth is low or nil in the 10 ‰ – 15 ‰ range with mortality occurring at salinities greater than 15 ‰. This agrees well with transplant experiments conducted in the Caloosahatchee that indicated mortality at salinities greater than 15 ‰ (Kraemer et al. 1999). Adair et al. (1994) found the distribution of V. americana in Trinity Bay, Texas limited to salinities less than 10 ‰. In outdoor mesocosm experiments, French (2001) observed minimal growth of *Vallisneria* from the Chesapeake Bay at 10 ‰ and 15 ‰.

The combination of results from field monitoring and laboratory experiments conducted by District and other investigators agree that 10 ‰ is a critical threshold salinity for growth. The thirty day averaging period is consistent with laboratory experiments which show that *Vallisneria* can survive exposure to 10 ‰ for periods exceeding a month (Doering et al. 1999; French 2001).

The daily average salinity limit of 20 ‰ was included in the rule to avoid acute exposure to high salinity. Laboratory experiments (1/5/1998, Table 1) showed that mortality occurs after a 20 day exposure to 18 ‰. Shorter exposure (1, 5, or 11 days) retarded growth but did not cause mortality (Doering et al. 2001). Recently completed experiments (5/20/2001, Table 1) suggest that a 50 % loss of shoots would occur after 16

days of exposure to 20 o/oo. At the earliest, mortality began after 3 days exposure to 20 ppt (One-way ANOVA,  $p < 0.05$ , **Figure D-4**). Therefore, a one day exposure to 20 ppt appears to be a reasonable limit for acute exposure.

Mean monthly flows less than 300 cfs are associated with low densities in the field (**Figure D-3**). These monitoring data indicate that 300 cfs should maintain *Vallisneria* in the upper Caloosahatchee estuary.

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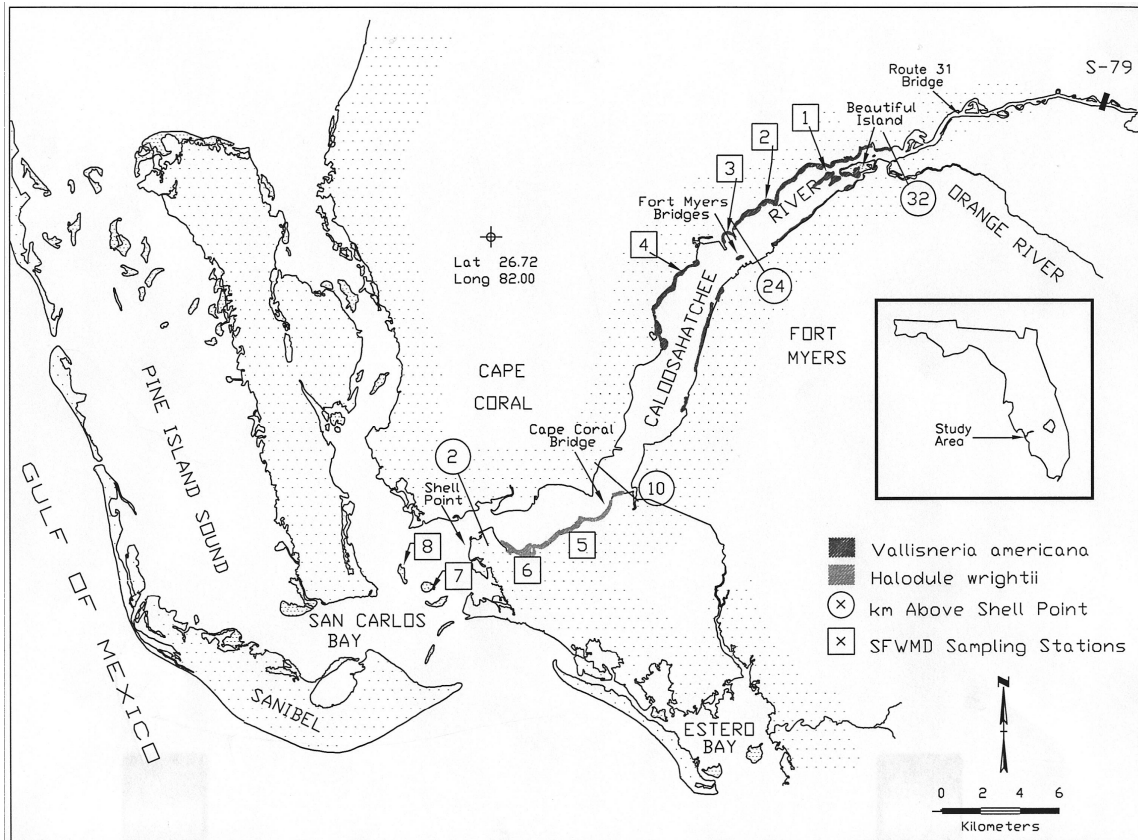
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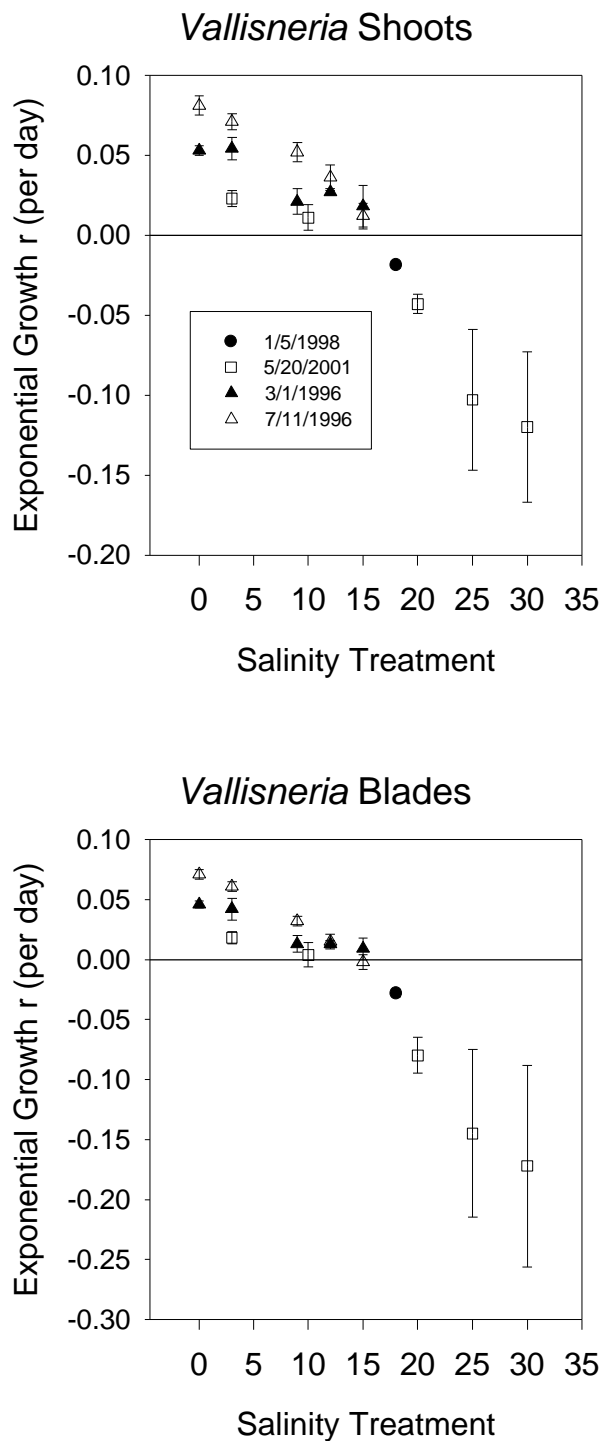
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Table 1. Summary of salinity tolerance experiments conducted with *Vallisneria americana*. Date refers to the beginning of an experiment. Exposure refers to the number of days plants were exposed to a given constant salinity treatment (‰).

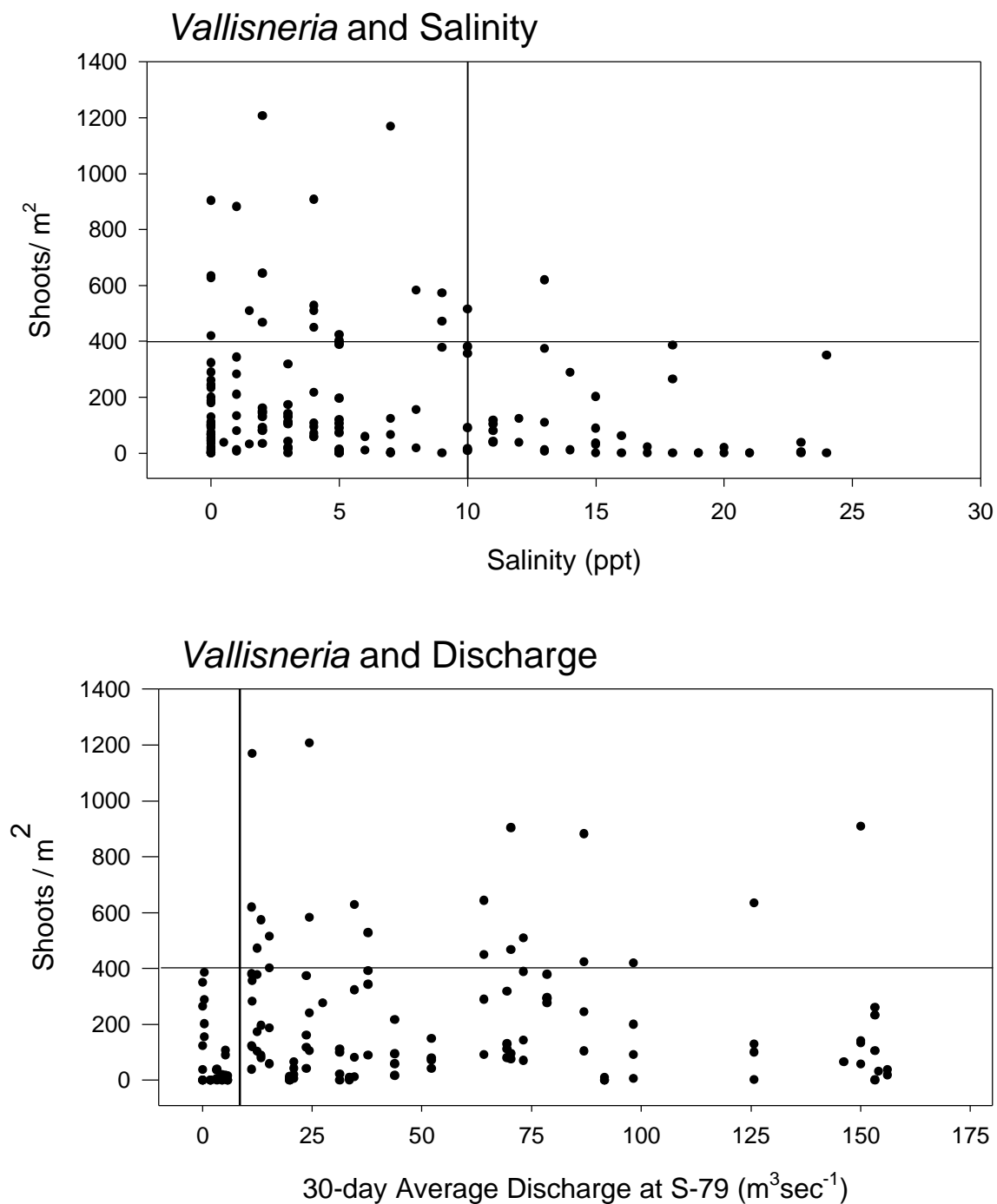
Species	Date	Salinity Treatments (‰)	Exposure (Days)
<i>Vallisneria</i>	3/1/1996	0, 3, 9, 12, 15	43
	7/11/1996	0, 3, 9, 12, 15	43
	1/5/1998	18	20, 30, 50, 70
	5/20/2001	3, 10, 20, 25, 30	36



**Figure D-1.** Distribution of *Vallisneria americana* and *Halodule wrightii* in the Caloosahatchee Estuary. Also shown are the locations of grass bed monitoring stations and the general locations of salinity recorders at S-79, Rt. 31 Bridge, Fort Myers Bridges, and Shell Point.



**Figure D-2.** Net exponential growth rates ( $r \pm 95\%$  C.I.) of *Vallisneria americana* measured in laboratory mesocosms during constant exposure to different salinities. A negative value of  $r$  indicates mortality.



**Figure D-3.** Shoot density of *Vallisneria americana* at monitoring stations 1, 2, 3 and 4 as a function of salinity on the day of collection (top panel) and average discharge at S-79 for the 30 days prior to the day of collection (bottom panel).

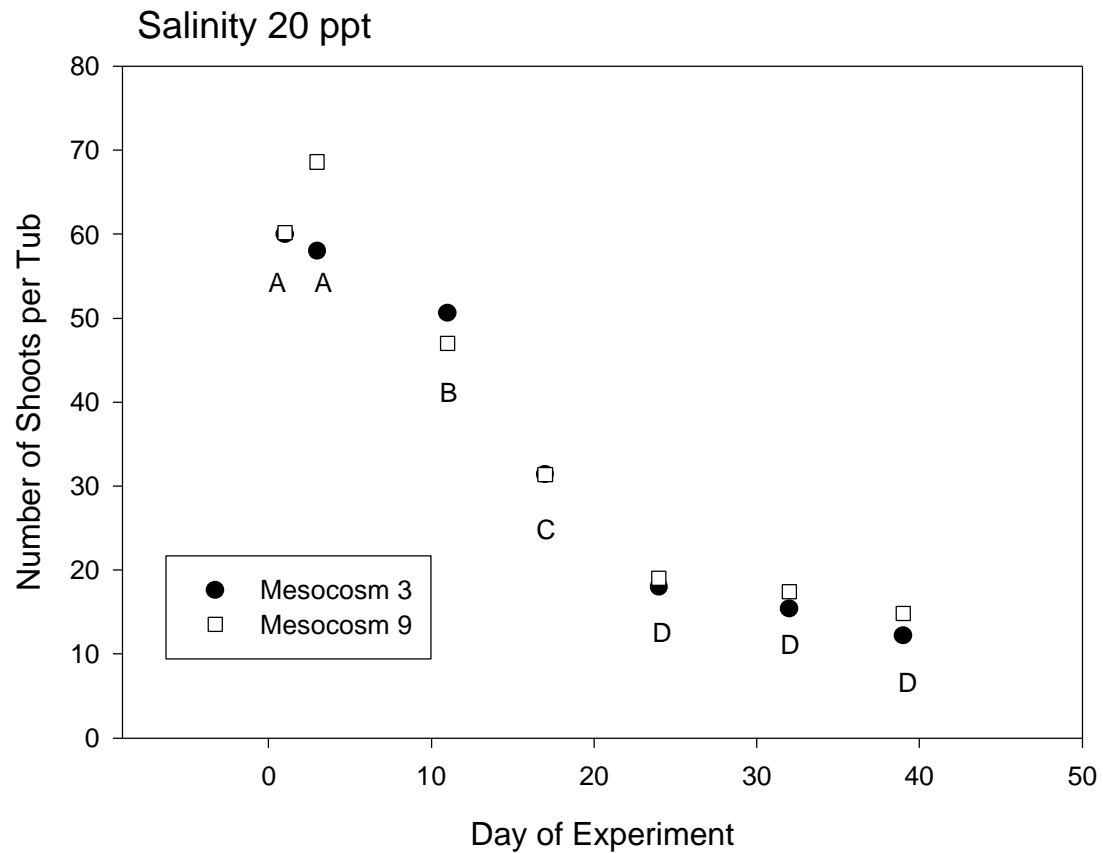


Figure D-4. Average number of shoots per tub in two replicate mesocosms exposed to 20 ppt. Letters indicate results of a comparison of daily means using the Student -Newman-Keuls test. Days with different letters are statistically different ( $p < 0.05$ ).